The Non symbiotic Origin of Mitochondria

The question of the origin of the eucaryotic cell and its organelles is reexamined.

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The greatest evolutionary discontinuity between living organisms is that separating procaryotic from eucaryotic cells. While there is ample biochemical evidence demonstrating that these two classes did not arise independently, the fundamental differences in their basic organization has made it extremely difficult to reconstruct their evolutionary history.

One of the most puzzling features of eucaryotic cellular organization is the existence of semiautonomous cytoplasmic genomes in such organelles as mitochondria and chloroplasts. The presence of these self-replicating organellar genomes, and the resemblance of the associated organellar systems of protein synthesis to bacterial systems has led to the wide acceptance of a theory originally propounded in the late 19th century that these organelles had their origin in a symbiotic association of bacteria and blue-green algae with the ancestral eucaryotic cells (*1*–*5*). This theory requires that various organelles were actually generated in several symbiotic events (*3*, *4*, *6*).

Since this view has gained wide popularity, we chose to reexamine the data used in its support with respect to the origin of mitochondria.

In our opinion there is no a priori reason why the eucaryotic cell, which has proved capable of remarkable evolutionary innovations, should have originated as a collage of procaryotic cells and parts of cells rather than have evolved in a more direct manner from a particularly advanced type of procaryotic cell. While symbiosis may have been of some evolutionary significance, overdependence on it as an explanation for the origin of the eucaryotic cell and its organelles may leave interesting questions unasked. Furthermore, dogmatic adherence to this theory leads to such improbabilities as the postulation of the origin of cilia from symbiotic spirochaetes (*1*, *3*, *4*), implying a nonexistent homology between flagellin and microtubule protein (*7*), and the multiple origin of chloroplasts from three entirely separate groups of photosynthetic procaryotes (two of them hypothetical) (*6*).

We shall show that while the symbiotic theory may be esthetically pleasing, it is not compelling, and we will propose an alternate hypothesis for the origin of the eucaryotic cell (*8*, *9*).

Outline of the Symbiotic Theory

The current symbiotic theory for the origin of the eucaryotic cell and its mitochondria is succinctly discussed by Stanier (*5*).

By this theory, as the primitive earth atmosphere began to change from anaerobic to aerobic as the result of photosynthetic oxygen production, procaryotes which had utilized a wide variety of anaerobic metabolic pathways were forced either to adapt to aerobic conditions or to become restricted to the few anaerobic environments remaining. Since eucaryotes are restricted to glycolysis for their anaerobic energy supplies, the ancestral protoeucaryote likewise utilized glycolysis. This protoeucaryote, by various adaptations, escaped from the selective pressure of free oxygen, the determinant driving the evolution of advanced oxidative metabolic pathways in other contemporaneous procaryotes. By evolution of larger cell size, intracellular translocation, advanced mechanisms for motility, and the ability to phagocytize, the protoeucaryote became able to ingest procaryotes as prey to provide substrates for glycolysis. Related and subsequent to these advances was the establishment of stable intracellular symbiotic relationships between the protoeucaryote and certain ingested aerobic procaryotes. Such relationships exist in present-day organisms. The terminal stage in eucaryote evolution was thus the acquisition of oxygen mediation (photosynthesis and respiration) by several quantum steps.

The theory further requires that in the course of time the symbiotic association has become extremely intimate. Most of the genetic information required for assembly of the organelle-symbiont has been transferred to the nuclear genome. The informational content of the organellar genome has been concomitantly much reduced and this genome as well as the organellar protein synthesis systems are evolutionary relicts.

This hypothesis has two particularly awkward aspects. The first is that the postulated protoeucaryote possessing many advanced cellular adaptations should have been so primitive and inefficient metabolically. In the face of competition from conventional procaryotes possessing more efficient aerobic, energy-yielding pathways already foreshadowing the patterns observed today, this should have left it at a considerable disadvantage. Second, the integration of the endosymbiont-proto-

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mitochondrion required wholesale transfer of genes from the endosymbiont genome to an unrelated nuclear genome. A mechanism by which this end may have been achieved is extremely difficult to conceive. Furthermore, the eucaryote fossil record and eucaryote biochemistry do not support the symbiotic theory.

Time of Appearance of Eucaryotes in the Precambrian

The symbiotic theory proposes that eucaryotes began their evolution as anaerobic cells which acquired aerobic symbionts as free oxygen began to appear. The time of appearance of eucaryotes in the fossil record does not bear out this part of the hypothesis, since eucaryotes do not appear in the fossil record until after atmospheric oxygen became available. Furthermore, the preservation of eucaryotes in stromatolites, produced by blue-green algae, suggests that they arose in a microenvironment that was aerobic even if the general level of oxygen in the atmosphere was too low to support aerobic metabolism at the time that eucaryotes began their evolution. Thus, eucaryotes have from the first been associated with free oxygen.

It is now generally accepted that the original atmosphere of the earth was anaerobic and contained simple reduced compounds of carbon and nitrogen, required for the evolution of life (*10*). The first organisms were anaerobic heterotrophs. These gave rise to a variety of anaerobic procaryotic heterotrophs and photosynthesizers. Fossil evidence indicates that blue-green algae producing oxygen as a photosynthetic by-product may have been in existence more than 2.5 billion years ago (*11*, *12*).

Photosynthetic production of oxygen was probably a major cause of the eventual replacement of the ancient atmosphere of the earth by one containing free oxygen (*13*; however, see also *14*). Berkner and Marshall (*15*) calculated that an oxygen concentration sufficient for aerobic metabolism coincided with the base of the Cambrian (about 600 million years before the present). However, Cloud (*13*, *16*) has noted that oxidized red beds appear in the geological record about 1.8 billion years ago, and proposes that these deposits mark the appearance of free oxygen in the atmosphere. The concentration of free oxygen may have been low until late in the Precambrian (*17*). The metazoa (including annelids and arthropods) appear in the fossil record at the end of the Precambrian (Ediacaran, about 650 million years in age) (*13*, *18*). Minimal concentrations of oxygen that would support the physiological processes of complex metazoa probably existed for a significant period of time prior to Ediacaran times and thus allowed for the evolution of these animals (*19*).

In the main, Precambrian fossils consist of the remains of microscopic procaryotic organisms or macroscopic traces of their activities. The most prominent macrofossils are the stromatolites which formed large reefs in the Precambrian (*18*, *20*). Recent stromatolites are formed in tropical regions by multispecific mats of filamentous blue-green algae growing in the intertidal zone (*21*, *22*). In Precambrian times stromatolites may well have grown in the subtidal zone from which they are now excluded because of competition and grazing (*22*). Blue-green algal mats would have provided an oxygen-rich environment shielded from high levels of ultraviolet light (*23*). Eucaryotes are present in the Late Precambrian Bitter Springs Formation of Australia (900 million years old), but absent from the ecologically similar Middle Precambrian Gunflint Iron Formation of Ontario (age 1.6 to 2.0 billion years) (*12*). The oldest microorganisms reasonably interpretable as eucaryote fossils are from the Beck Springs Dolomite of California (1.2 to 1.4 billion years of age) (*17*, *24*). These occurrences are therefore at least 400 million years younger than the red beds that indicate the beginning of free oxygen accumulation in the atmosphere.

Eucaryotic Cells Are Basically Aerobic

Several fundamental biochemical pathways of the eucaryotic cytoplasm indicate a primitive adaptation to the use of oxygen. A consideration of these systems will show that the eucaryotic cell is not simply an anaerobic cytoplasm containing an aerobic respiratory organelle. This difficulty for the symbiotic theory has also been pointed out by Cohen (*25*) in his interesting discussion of the origin of mitochondria from a biochemist's viewpoint.

Enzymic protection from oxygen toxicity. Autoxidation of a number of cell components produces the superoxide radical O_2^-. This highly reactive ion is destroyed by the enzyme superoxide dismutase, which catalyzes the reaction:

$$O_2^- + O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

This enzyme and catalase appear to be ubiquitous in aerobes, and are probably vital to the existence of organisms metabolizing oxygen (*26*). It is significant that the bacterial superoxide dismutase appears to be quite different from the mammalian enzyme (*26*). Catalase and other oxygen detoxifying enzymes of eucaryotes are packaged in specialized organelles, called peroxysomes (*27*). Superoxide dismutase apparently occurs both free in the cytoplasm and bound to as yet uncharacterized particles (*28*). Peroxysomes are found in such evolutionary diverse cells as *Tetrahymena*, yeasts, higher plants, and mammalian liver and thus may, as pointed out by DeDuve, represent an organelle evolved by primitive eucaryotes for protection from oxygen.

Requirements of anaerobic eucaryotes. While anaerobic procaryotes exist in considerable diversity, there are few anaerobic eucaryotes. The largest group of anaerobic eucaryotes is composed of flagellated protozoa inhabiting anaerobic environments in the intestinal tract of animals. Examples of these are the trichomonads (which lack mitochondria) (*29*) and the rumen protozoa (*30*). There is also an obligate anaerobic fungus, *Aqualinderella*, which lives on submerged fruit (*31*). Stanier (*5*) suggests that these organisms are not primitively anaerobic but are secondarily adapted to specialized niches.

Because much is known about the metabolism of yeast, it is of interest to note that yeast can be grown anaerobically, but only if provided with oleate and a steroid (*32*). Oleate and steroids require the presence of oxygen for their biosynthesis and are synthesized by yeast grown aerobically (*33*). Thus yeast, a primitive eucaryote, ultimately has an absolute requirement for oxygen (*34*). The various anaerobic eucaryotes discussed above may well have similar requirements which are met by their close association with aerobic eucaryotes.

Oxygen and biosynthetic patterns. Bloch (*35*), and Goldfine and Bloch (*36*) have discussed in detail the relationship of oxygen to biosynthetic patterns and have made two very significant points. First, components universal to all cells are not invariably synth

sized by a common pathway. A number of compounds are synthesized anaerobically by some organisms and aerobically by others. Second, products of some oxygen-requiring pathways are unique to aerobic organisms. They are thus metabolic specializations superimposed on the ancient, common metabolic plan. Alternate aerobic and anaerobic pathways exist for monounsaturated fatty acids, tyrosine, nicotinic acid, carotenoids, and porphyrins. Steroids and polyunsaturated fatty acids have no anaerobic pathways. Particularly significant with regard to the hypothesis of a primitively aerobic eucaryotic cell are the pathways for unsaturated fatty acids and steroids. Eucaryotes and some advanced procaryotes use an aerobic pathway for monounsaturated fatty acids. Eubacteria, whether aerobic or anaerobic, utilize an entirely unrelated anaerobic pathway. Bloch (*35*) proposes that the change in pathway occurred during the evolution of advanced procaryotes and was retained by eucaryotes. The proposed selective advantage was that the monounsaturated fatty acids produced aerobically (for example, oleic acid) serve as substrates for the production of certain polyunsaturated fatty acids (linoleic and more highly unsaturated fatty acids). Polyunsaturated fatty acids are absent in both aerobic and anaerobic bacteria but are universal in eucaryotes. Steroids which are of universal occurrence in eucaryotes have been recently detected in procaryotes as well (*37*). The lack of anaerobic pathways for steroids as well as their universal occurrence in eucaryotes suggests that this aerobic pathway was present in the ancestral eucaryote.

Table 1. Properties of mitochondrial protein synthesis systems which are divergent from procaryotic protein synthesis systems.

Site	System			References
	Bacteria	Ascomycete mitochondria	Animal mitochondria	
		Sedimentation coefficient (S)		
Ribosome	70	70–74	50–60	(*47, 79–81*)
Large subunit	50	50–58	33–45	
Small subunit	30	35–40	25–35	
		Molecular weights of rRNA		
Large subunit	1.10×10^6	$1.23–1.28 \times 10^6$	$0.65–0.95 \times 10^6$	(*47, 79*)
Small subunit	0.56×10^6	$0.63–0.79 \times 10^6$	$0.36–0.50 \times 10^6$	
		Guanine plus cytosine (percent of rRNA)		
Large subunit	52–53	25–34	38–46	(*47, 79, 81*)
Small subunit	52–54	27–38		
		Presence of 5S RNA		
Ribosome	Yes	No	No	(*82, 83*)
		Methylation of rRNA		
	Yes	?	Unsettled	(*47, 48, 79, 84*)
		Subunit exchange with bacterial ribosomes		
	Yes (various bacteria)	No	?	(*48, 85*)
		Inhibition of protein synthesis by fusidic acid		
	Yes	No	?	(*48, 50*)
		Effect of 120 mM NH_4Cl		
	Maximal stimulation (*E. coli*)	Inhibition (90 percent)	?	(*48, 85*)

The Near Ubiquity of Cytochromes

All organisms, aerobic and anaerobic (with the exception of the Clostridia), contain cytochromes (*38*). Aerobic bacteria possess cytochrome respiratory chains similar in function to the mitochondrial cytochromes (that is, cytochrome types b, c, and a). However, there are significant differences which suggest a considerable amount of evolutionary divergence between mitochondrial and bacterial cytochromes (*39*). Bacterial electron transport systems do not respond as does the mitochondrial system to some of the generally used inhibitors of mitochondrial electron transport (*40, 41*). Furthermore, the bacterial cytochromes are more varied than are the mitochondrial cytochromes (for example, c-type cytochromes include not only c and c_1, but also c′, c_2, c_3, c_4, and c_5), and there are several terminal oxidases (for example, a_1, a_2 that is now called d, and o, as well as $a + a_3$, the oxidase of mitochondria). Some bacteria have only one oxidase, others have two or three (*40*). Multiple oxidases are particularly prevalent in cells capable of adapting to different oxidants. Mitochondrial cytochrome c interacts very poorly with most bacterial cytochrome oxidases and vice versa. Isolated bacterial c-type cytochromes are different from mammalian cytochrome c in primary sequence and in such properties as isoelectric point and redox potential, although they have a common prosthetic group (*39, 40, 42*). In common with mitochondrial cytochromes, bacterial cytochromes are membrane bound, and cytochrome c is the most readily extracted cytochrome (*40*).

Also significant is the widespread occurrence of cytochromes in anaerobic bacteria in which they function in electron transport between organic substrates or molecular hydrogen and a variety of inorganic oxidants (*38, 43*). Striking also is the persistence of hemoproteins called P450, the terminal oxidase in hydroxylase (mixed function oxidase) reactions throughout all current forms including bacteria (*39*). In eucaryotes P450 is generally associated with the microsomal and not the mitochondrial fraction. These observations suggest that rather than being the exception among Precambrian procaryotes, cytochrome electron transport chains were probably the rule. When oxygen began to become available as an electron sink, many organisms were able to modify their cytochrome systems to utilize oxygen. There is no a priori reason for us to assume it was otherwise with the cells ancestral to eucaryotes.

Relatedness of Eucaryotic and Procaryotic Cytochrome c Sequences

The sequence homology between eucaryotic and procaryotic c-type cytochromes indicates that these are related evolutionarily (*44*). Eucaryotic cytochrome c is localized and functions in the mitochondrion, yet the gene for cytochrome c resides in the nucleus (*45*). This situation arose either by transfer of this gene from the genome of the endosymbiont protomitochondrion to the nuclear genome, or by the gene for cytochrome c always having been present in the nuclear genome wherein it underwent its evolution.

Proponents of the symbiotic theory maintain that the permanent establishment of the endosymbiont was a late

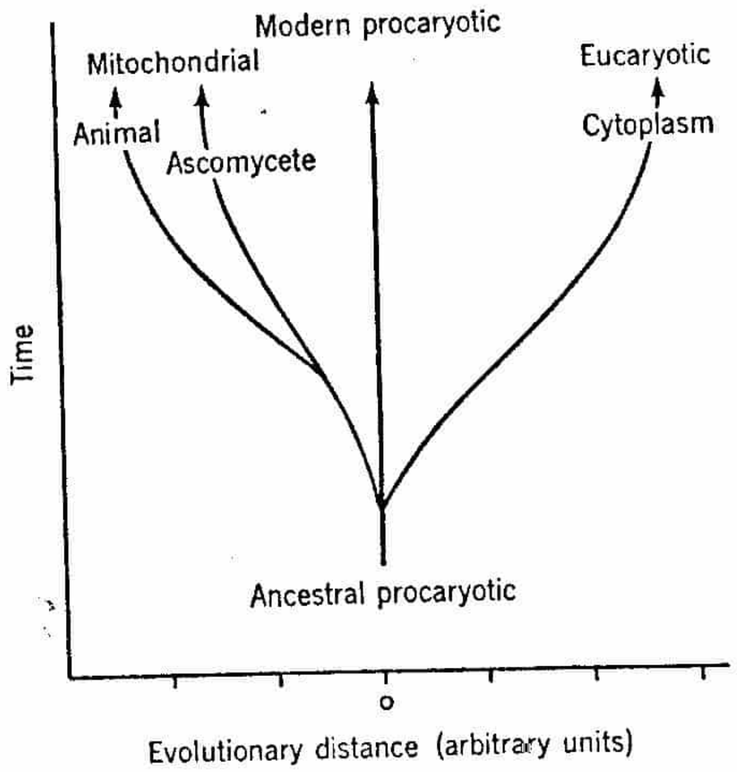

Fig 1. Evolution of ribosomes: a simplified phylogeny of mitochondrial, eucaryotic and procaryotic ribosomes. For simplicity, it is assumed that modern procaryotic ribosomes are identical to those of Precambrian procaryotes. Organellar (mitochondrial) and eucaryotic cytoplasmic ribosomes have diverged in various ways from the basic procaryotic pattern. Mitochondrial ribosomes have diverged less than cytoplasmic ribosomes, but show significant differences from procaryotic ribosomes and from each other.

event in the evolution of the eucaryotic cell. In agreement with this, many of the constituents of the mitochondrion are indeed quite procaryote-like. Therefore, it is reasonable to expect significant similarities between eucaryotic and procaryotic cytochromes c. A measure of this relatedness has been provided by McLaughlin and Dayhoff (*46*). They found that the degree of divergence between sequences in eucaryotic and procaryotic cytochromes c is comparable to that between sequences in eucaryotic and procaryotic transfer RNA (tRNA). These measures of divergence both for tRNA and for cytochrome c between eucaryotic and procaryotic cells are significantly greater than between various eucaryotic kingdoms. This suggests that the gene for cytochrome c has resided in the nuclear genome since the beginning of the divergence of eucaryote from procaryote cells. It is not in agreement with a late acquisition of a bacterial gene for cytochrome c.

Mitochondrial Protein Synthesis

The undeniable fact remains that the mitochondrion (as well as the chloroplast) contains its own genome and its own protein synthetic apparatus: both different from and apparently unrelated to the nuclear-cytoplasmic system. The protein synthesis systems of mitochondria have a strong resemblance to those of procaryotes. However, this resemblance has been overstated, and mitochondrial protein systems have several unique features (Table 1). The most apparent divergences between mitochondrial and bacterial ribosomes are structural. Ascomycete mitoribosomes are somewhat larger than bacterial ribosomes and contain species of ribosomal RNA (rRNA) having higher molecular weights. On the other hand, animal mitoribosomes are much smaller than bacterial ribosomes and constitute the smallest structures responsible for protein synthesis. Mitochondrial rRNA's are peculiar in several respects; they are very low in guanine plus cytosine content and apparently are unmethylated. Furthermore, while a 5*S* (120 nucleotides) rRNA occurs in both bacterial and eucaryotic ribosomes, such an RNA is undetectable in mitoribosomes.

Mitoribosome subunits do not exchange with those of bacterial ribosomes. On the other hand, mitochondrial and bacterial initiation factors and polypeptide elongation factors appear to be interchangeable (*47*, *48*). Mitoribosomes share with bacterial ribosomes a sensitivity to chloramphenicol, and to several other inhibitors which do not inhibit eucaryotic cytoplasmic protein synthesis. Further, drugs such as cycloheximide, emetine, and anisomycin which inhibit eucaryotic ribosomes do not inhibit either bacterial or mitochondrial protein synthesis. Interestingly, fusidic acid which inhibits both bacterial and eucaryotic protein synthesis (*49*) has been reported to have no effect on mitochondrial protein synthesis in *Neurospora* (*50*).

Initiation of protein synthesis in mitochondria and bacteria is very similar (*47*, *51*). Mitoribosomes can interact with bacterial initiation factors F1 and F2. Furthermore, both utilize formylmethionyl-tRNA in response to the initiation codon AUG (adenine, uridine, guanine). Formylmethionyl-tRNA (fMet-tRNA^{fMet}) initiates polypeptide chains in the mitochondrion but it is not the initiating tRNA in the eucaryotic cytoplasm (*52*).

However, two species of methionyl tRNA do exist in the cytoplasm of eucaryotic cells, tRNA^{Met} and tRNA^{fMet*} (uncharged) (*51*, *53*). Methionyl tRNA^{fMet*} can be formylated in vitro by *Escherichia coli* transformylase. Formylmethionyl-tRNA^{fMet*} derived in this way from yeast can initiate protein synthesis in extracts of *E. coli*, and con-

Table 2. Intracellular location of genes for proteins of mitochondrial DNA replicative, RNA synthetic, and protein synthetic systems.

Protein	Function and properties	Gene location	References
DNA polymerase (mammalian, ascomycetes)	Polymerase distinct from nuclear enzyme	Nuclear	(*81*, *86*, *87*)
RNA polymerase (*Neurospora*)	Rifamicin sensitive and amanitin insensitive, therefore, bacteria-like; single polypeptide: 64,000 daltons (smallest known polymerase) *	?	(*71*, *88*)
(Yeast)	Sensitive to ethidium bromide	Nuclear	(*75*, *89*, *90*)
(HeLa)	Symmetrical transcription†; sensitive to ethidium	?	(*48*, *75*)
Ribosomal proteins (*Neurospora*)	30 in large subunit; 23 in small subunit; all distinct from cytoplasmic ribosome proteins	Nuclear	(*47*, *91*, *92*)
Erythromycin-resistance factor (yeast)	A ribosomal protein or perhaps a modified RNA	Mitochondrial	(*93*, *94*)
Polypeptide chain initiation factors (*Neurospora*)	Bacteria-like	?	(*47*)
Polypeptide chain elongation factors (yeast)	Factors required for mitochondrial protein synthesis can substitute for bacterial elongation factors with bacterial ribosomes	Nuclear	(*47*, *95*)

* Bacterial polymerase consists of several polypeptide chains. † This is unique; bacterial and nuclear transcription are asymmetrical.

versely *E. coli* $tRNA^{fMet}$ can initiate hemoglobin synthesis in vitro (*53*). Nonformylated methionyl $tRNA^{fMet*}$ is the natural initiator in the eucaryote cytoplasm (*53, 54*). The initiation mechanisms of the eucaryote cytoplasm probably originally used fMet-$tRNA^{fMet}$ and in fact the changes from the procaryotic pattern have been evolutionarily conservative (loss of transformylase, conversion of $tRNA^{fMet}$ to $tRNA^{fMet*}$). Regardless of the origin of the two synthetic systems responsible for protein synthesis in the eucaryotic cell, both have been derived from an ancestral procaryotic system. During their long coexistence in the cell these systems have been part of two different units of selection [in the sense discussed by Lewontin (*55*)], and have diverged both from the ancestral procaryotic pattern and from each other. Thus both mitoribosomes and cytoribosomes show similarities to and dissimilarities from the procaryotic ribosome. It is more significant that the mitoribosomes of animals are greatly divergent from those of ascomycetes and neither can really be called typically "bacterial" (Fig. 1). Two other aspects of mitochondrial protein synthesis bear examination: (i) In which genome are the genes for the mitochondrial ribosomes located? (ii) What is the evolutionary advantage of maintaining a mitochondrial protein synthetic system?

Mitochondrial rRNA is unquestionably coded for in the mitochondrion (*48*). On the other hand most if not all of the mitochondrial ribosomal proteins are coded for by the nucleus and synthesized on cytoplasmic ribosomes (Table 2). Yet the mitochondrial ribosomal proteins are quite distinct from cytoplasmic ribosomal proteins which are also coded for by the nucleus. The question of the evolutionary advantage of maintaining two distinct systems for protein synthesis is difficult to answer. Nevertheless, such an advantage certainly exists since mitochondrial protein synthesis (regardless of its evolutionary origin) has been retained by eucaryotic cells from yeast to man. Interference with mitochondrial protein synthesis prevents the formation of mitochondria capable of respiration (*56, 57*) and is thus lethal unless the cell is capable of fermentative growth (for example, yeast). Yet the products of mitochondrial protein synthesis are few in number—there are a few polypeptides (function unknown) of the inner membrane, an attachment site for adenosine triphosphatase (*58*), and probably a portion of cytochrome oxidase (*48, 57, 59*). The maintenance of an elaborate extranuclear genetic system throughout the approximately 1.2 billion years of eucaryote evolution for the synthesis of these few proteins indicates the existence of peculiar and severe constraints on the site of synthesis and assembly of certain mitochondrial components. Why these proteins should not be synthesized on cytoribosomes and then transported to their assembly site is still unknown, but may be related to the peculiar topology of the mitochondrion or to the hydrophobic nature of the proteins which may make them too insoluble for transport.

That a vital role for mitochondrial protein synthesis has been retained suggests to us that this is not merely a relict function of an originally endosymbiotic organelle. Especially cogent in this regard is the fact that much of the mitochondrial ribosome is encoded in the nucleus and that an apparently irreducible part is coded for by the mitochondrial genome. We suggest that certain elements of the protoeucaryote's respiratory membrane were synthesized and assembled in situ (Fig. 2). As long as this membrane was exposed to cytoplasm, ribosomes and messenger RNA (mRNA) could reach the site of assembly. The ribosomes may well have been membrane bound at the site of synthesis (*60*). Segregation by binding to membranes, of ribosomes synthesizing particular classes of proteins, has been proposed by Tata (*61*).

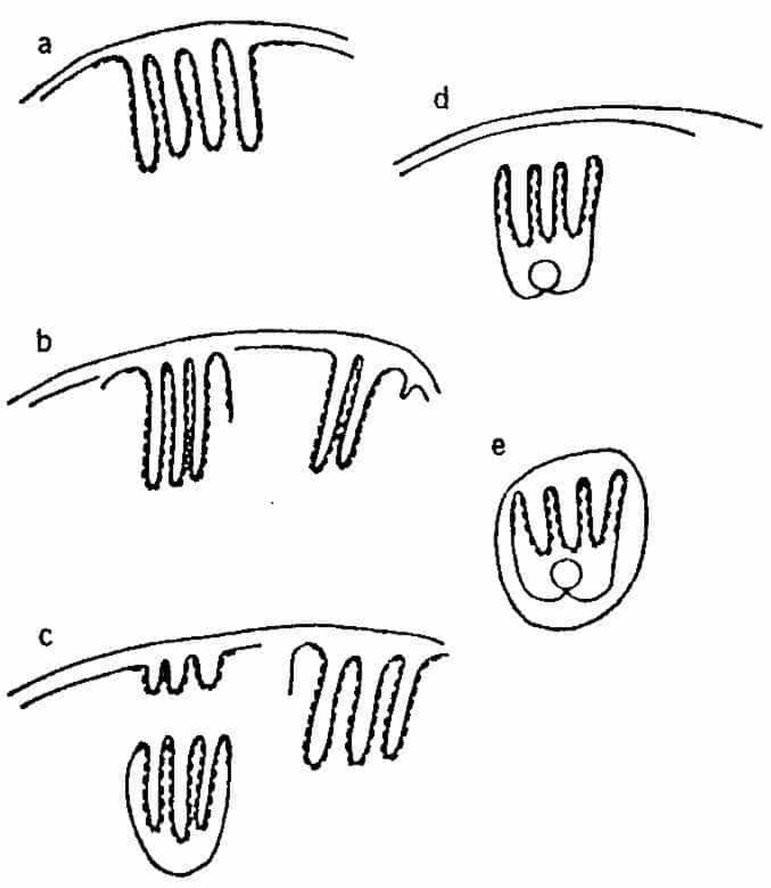

Fig. 2. A schematic representation of the origin of mitochondria from a simple procaryotic respiratory organelle. The drawings present cross sections of hypothetical cells representing various evolutionary stages. Blocks on the membrane represent respiratory assemblies. (a) Section of protoeucaryote showing invaginated cell membrane possessing respiratory function. (b) As the protoeucaryote becomes large, a more extensive respiratory surface becomes necessary, and is provided by blebbing off of respiratory membranes from the cell membrane. (c) Topologically closed respiratory organelles generated by blebbing. (d) Establishment of a stable plasmid (schematically represented by a circle) containing genes for ribosomal components and some elements of the respiratory membrane. (e) The final step in the evolution of the mitochondrion in the later acquisition of an outer membrane.

The Origin of the Mitochondrion: A Model

While the symbiotic model assumes an anaerobic protoeucaryote that acquired a respiratory endosymbiont, we propose that the protoeucaryote was an advanced, aerobic cell rather larger in size than is typical for procaryotes. This trend to larger size necessitated [concomitantly with many of the changes in cellular organization discussed by Stanier (*5*)] a large increase in respiratory membrane surface. This was achieved initially by invagination of the inner cell membrane (Fig. 2a), and later by formation of membrane-bound vesicles generated from the inner cell membrane (Fig. 2, b and c). The respiratory particles thus generated were topologically closed objects surrounded by a membrane providing a selective permeability barrier between the respiratory elements and the cytoplasm. This was evolutionarily advantageous, and was the basis for a more sophisticated regulation of respiratory metabolism (*41*). However, this permeability barrier posed a problem to the cell since certain constituents of the respiratory chain (for example, elements of cytochrome oxidase) required synthesis in situ. While the membrane surrounding the respiratory elements was permeable to many proteins, including cytochrome c and enzymes of the respiratory and phosphorylation sequences, it was impermeable to ribosomes or ribosomal RNA. Thus, the respiratory organelles required constant de novo replacement from the cell membrane.

Because such constant turnover of this complex organelle was uneconomical, it would have been a considerable advantage for these cells to implant a system for protein synthesis on the inside of the organelle for organelle maintenance. While this seems a formidable problem on first sight, in fact it need not have involved anything extraordinary at all. We propose that the cell implanted a protein synthesis

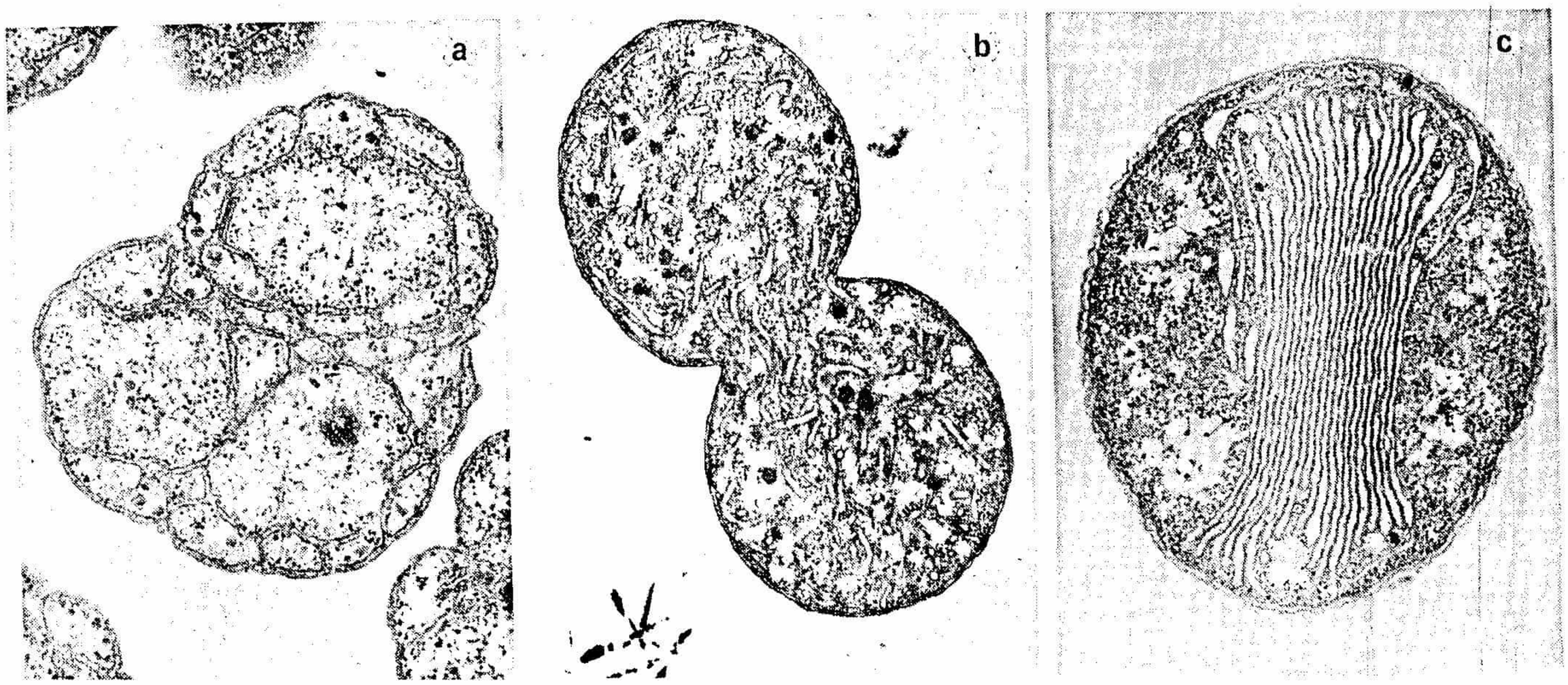

Fig. 3. Complex internal membranes of bacteria; (a) *Nitrosolobus multiformis*, (b) *Nitrococcus mobils*, (c) *Nitrosococcus oceanus* [see (*101*)]. [Courtesy of Dr. S. Watson, Woods Hole Oceanographic Institute]

system into the respiratory organelle by simply incorporating a stable plasmid containing the appropriate genes for ribosomal components (Fig. 2d). An analogous process occurs in the generation of multiple nucleoli during amphibian oogenesis. In that process, multiple replicates (circular in configuration) of the ribosomal genes are made from the chromosomal rRNA genes and are packaged in free nucleoli which produce the large amount of rRNA required by the egg (*62*). The hypothetical respiratory organellar plasmid of the protoeucaryote may not, of course, have been generated by the same mechanism, but this example serves to show that the proposed organellar plasmid is not in the least farfetched, and has an existing counterpart (see discussion of plasmids below).

Elements of the Model

Organelles of procaryotes. While the organelles of eucaryotic cells are certainly more complex (and better studied) than the organelles of procaryotic cells, it should be realized that procaryotes contain several types of membrane-bound organelles (for example, chlorobium vesicles, gas vesicles, thylakoids, and mesosomes) (*5*, *41*, *63*). Some are extensive and intricate as, for example, the internal membrane systems (mesosomes) shown in Fig. 3. The functions of such membrane systems are in many cases problematical, but there is good evidence that mesosomes perform two functions which make them particularly pertinent to the model being advanced.

1) Mesosomes apparently contain the respiratory membranes of bacteria and are thus mitochondrial equivalents (*40*, *41*). They are the site of various reductase activities (*64*), and Ferrandes *et al.* (*65*) who isolated mesosomes and cytoplasmic membranes from *Bacillus subtilis*, found that the cytochromes were preferentially localized in the mesosomal fraction.

2) Replication of the bacterial chromosome and of plasmids apparently involves a membrane-bound replication site (*41*, *66–69*). It has been proposed that the replication sites are associated with mesosomes (*67*).

Thus the procaryotic cell possesses a respiratory organelle equivalent to the mitochondrion in general function. This organelle would have been available in the protoeucaryote for evolutionary modification. Further, this organelle already possessed a site for plasmid replication which would have been available for attachment of the hypothetical plasmid of the model (Fig. 2d).

Plasmids. Extrachromosomal genomes (called plasmids or episomes) occur widely among procaryotes (characteristics of plasmids are summarized in Table 3). Several properties of plasmids support the idea that they are ancestral to the mitochondrial genome. They are similar in size to mitochondrial DNA's, and share the property of being supercoiled circles and of being subject to elimination by acridines and ethidium. Plasmids, like the chromosomes, are capable of autonomous self-replication in a bacterial cell. Replication of plasmids involves both plasmid-linked and chromosome-linked genes. Further, plasmid replication seems to involve specific replication sites—perhaps on the cell membrane. Plasmids, however, contain not only genes required for their replication, but also a variety of other genes. Those plasmids carrying such characters as sex factor or various drug resistance factors have been well studied. Of particular significance to the proposed model is the fact that plasmids are capable of direct genetic interaction with the chromosome by way of integration into the chromosome. This integration seems to involve insertion of plasmid DNA, by utilization of the cell's recombination enzymes, into a region of the chromosome possessing some homology with a region of the plasmid. Integration is often reversible, and excision of the plasmid in some instances involves excision of chromosomal genes so that a novel plasmid is generated (Fig. 4). These events are not particularly rare, and in a natural population subject to certain selection pressures plasmids bearing advantageous genes very quickly become apparent. This property of plasmids has been of special significance with respect to antibiotic resistance, since plasmids carrying several antibiotic resistance genes have been found. The evolutionary flexibility of this genetic mechanism is particularly great since such plasmids are transmissible from one bacterium to another. Thus there is a continuous flow of genetic information between chromosomes and plasmids. We propose that incorporation into plasmids

is not restricted to genes for antibiotic resistance or for certain metabolic enzymes, but can or has been extended to genes for rRNA, tRNA, and various membrane proteins. In fact, genes for tRNA are found in one class of plasmids, the temperate bacteriophages (*70*). Given a selective pressure on the protoeucaryote for the incorporation of a protein synthesis system into its respiratory organelle, generation of a plasmid with the appropriate genes would have been an efficient way to do so by exploiting the plasmid-nuclear interactions already established in the cell.

Genes for mitochondrial gene expression. The properties of the mitochondrial systems for DNA replication, RNA synthesis, and protein synthesis are quite distinct from the corresponding nuclear-cytoplasmic systems. Yet, with the notable exception of rRNA's and tRNA's, these systems are largely encoded by nuclear genes (Table 2). This is significant, because it so much resembles the situation of the respiratory chain which is also largely encoded by the nucleus (*9, 45, 48, 56, 57*).

According to the symbiotic model this situation arose by transfer of these genes from the endosymbiont to the nucleus. According to the nonsymbiotic model that we propose, these genes resided in the nucleus from the first, and the plasmid contained only the minimal number of genes needed for its replication and function after its sequestration into a closed organelle.

Direct support for such a contention is, unfortunately, not easily found; the most suggestive evidence comes from the peculiarly divergent properties of gene expression in mitochondria. As shown in Table 2, the transcriptive system of the mitochondrion has several features not found in either nuclear or bacterial transcription. In particular, the mitochondrial RNA polymerase of *Neurospora* has been isolated and characterized (*71*). It resembles eucaryotic nucleolar RNA polymerase and bacterial RNA polymerase in its insensitivity to α-amanitin which is a potent inhibitor of the principal eucaryotic nuclear RNA polymerase (II) (*72–74*). Like bacterial RNA polymerase the mitochondrial polymerase is sensitive to rifamicin. However, instead of being a large complex composed of several polypeptide chains, it is a single polypeptide with a molecular weight of about 64,000, and is thus the smallest known polymerase. It is not known if the gene for this enzyme is nuclear or mitochondrial, but there is suggestive evidence that the gene for yeast mitochondrial RNA polymerase is nuclear (*75*). The transcription process of mitochondria (at least of mammalian mitochondria) is also unique. Transcription in the HeLa mitochondrion is symmetrical, while bacterial and nuclear transcription is asymmetrical (*76*). Only one of the transcripts in the mitochondrion is actually utilized: the transcript of the other strand is degraded. The component proteins of the mitochondrial protein synthesis system are also all or nearly all nuclear gene products (see Table 1).

We suggest that the peculiar melange of bacteria-like and uniquely mitochondrial properties observed are an evolutionary product of two different inputs. First, both the nuclear-mitochondrial and the nuclear-cytoplasmic systems of interactions arose in a procaryotic cell and thus the divergent evolution of these two systems utilized the same starting material. In many ways the nuclear-mitochondrial system has been more conservative. Second, the regulatory requirements of the two systems are probably quite different, and the simplest way to manage these controls is to utilize separate components. Thus one finds different mitochondrial and nuclear RNA polymerases—although the genes for both may in fact be nuclear. Precedent for this hypothesis comes from the observation of Roeder and Rutter (*74*; see also *73, 77*) that there are at least two different RNA polymerases in the nucleus. One in the nucleoplasm is probably specific for the transcription of mRNA and the other in the nucleolus is specific for the transcription of rRNA.

The best supportive evidence for this

Table 3. Characteristics of plasmids.

Characteristic	Observations	References
Molecular weights	1.5×10^6 to 1.0×10^8 daltons	(*68*)
Conformation	Circular duplex, supercoiled	(*68, 69*)
Distribution	Eubacteria, photosynthetic bacteria	(*68, 96*)
Amount of plasmid DNA per cell	Varies with plasmid and host; may be 1 to > 30 copies per cell or up to 40 percent of total cell DNA	(*68, 97*)
Replication	Probable specific replication points on membrane; plasmid stability mutants linked with both plasmid and chromosome	(*66, 68, 69, 98, 99*)
Genomic content of varied plasmids	Replication genes, sex factor, colicinogenic factors, variety of genes for antibiotic resistance, various genetic markers excised from bacterial chromosome (lac, gal, trp, cysB)	(*69, 98, 100*)
Effect of acriflavine and other acridines, ethidium bromide	Plasmid eliminated from host	(*69*)

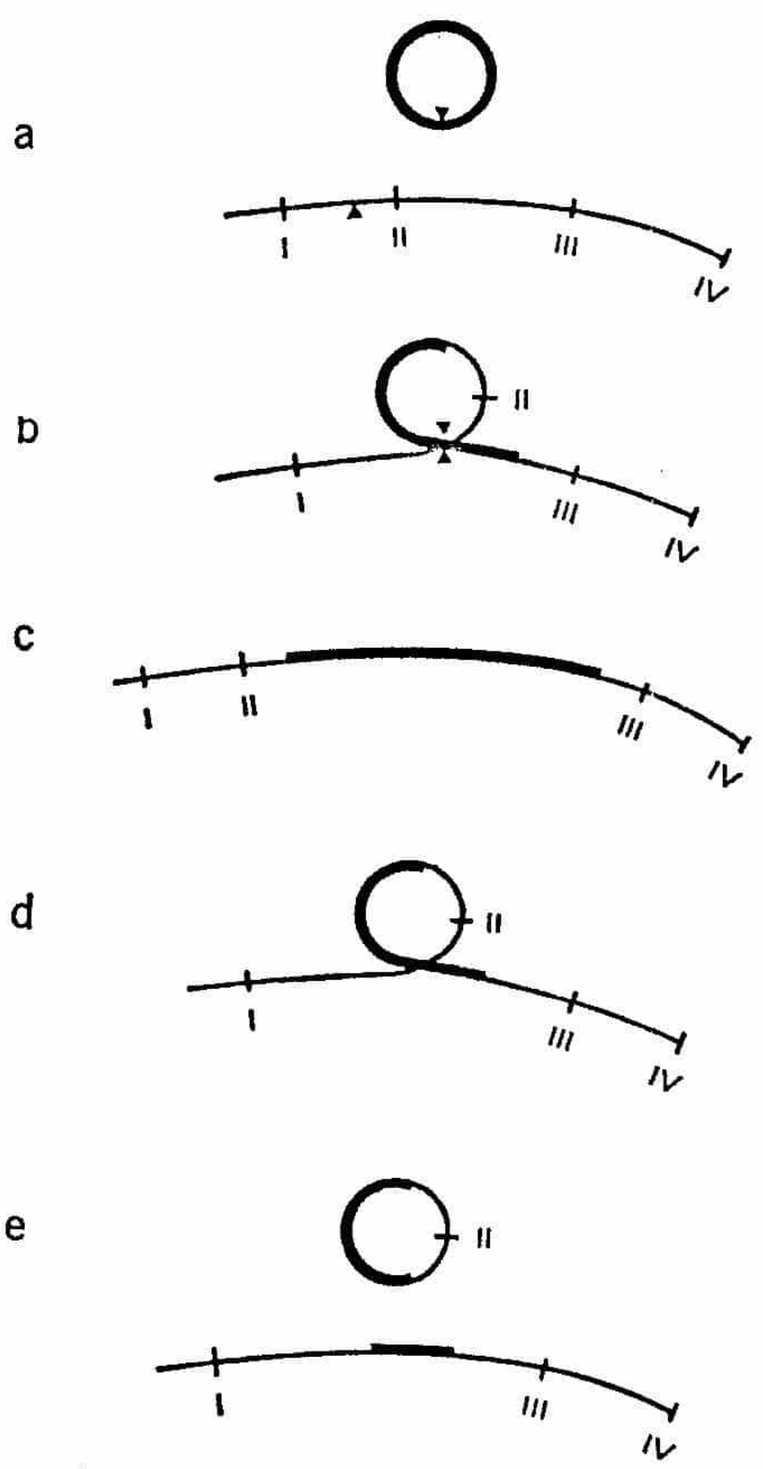

Fig. 4. Model for the reversible integration of a plasmid into a bacterial chromosome [model of Campbell, redrawn from (*69*)]. (a) Plasmid (heavy line) and chromosome (thin line) surviving independently in the same cell. (b) Apposition of homologous regions of plasmid and chromosome followed by single cross-over. (c) Integrated plasmid. (d) Apposition of homologous regions again followed by crossing-over. (e) Plasmid excised with incorporation of part of the chromosome carrying marker to yield a novel plasmid. By the proposed model such events occurred in the protoeucaryote and the marker may have been, for example, a gene for rRNA.

model may come from investigations of the possible role of plasmids in the generation of complex respiratory or photosynthetic membranes in procaryotes. Gibson (*78*) has already suggested that extranuclear genomes may play a role in the function of the photosynthetic organelles of procaryotes, though evidence for this is still lacking.

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